

PRIMARY RESEARCH ARTICLE

Colonizations cause diversification of host preferences: A mechanism explaining increased generalization at range boundaries expanding under climate change

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Abstract

As species' poleward range limits expand under climate change, generalists are expected to be better colonists than specialists, extending their ranges faster. This effect of specialization on range shifts has been shown, but so has the reverse cause-effect: in a global meta-analysis of butterfly diets, it was range expansions themselves that caused increases in population-level diet breadth. What could drive this unexpected process? We provide a novel behavioral mechanism by showing that, in a butterfly with extensive ecotypic variation, Edith's checkerspot, diet breadths increased after colonization events as diversification of individual host preferences pulled novel hosts into population diets. Subsequently, populations that persisted reverted toward monophagy. We draw together three lines of evidence from long-term studies of 15 independently evolving populations. First, direct observations showed a significant increase in specialization across decades: in recent censuses, eight populations used fewer host genera than in the 1980s while none used more. Second, behavioral preference-testing experiments showed that extinctions and recolonizations at two sites were followed, at first by diversification of heritable preference ranks and increases in diet breadth, and subsequently by homogenization of preferences and contractions of diet breadth. Third, we found a significant negative association in the 1980s between population-level diet breadth and genetic diversity. Populations with fewer mtDNA haplotypes had broader diets, extending to 3–4 host genera, while those with higher haplotype diversity were more specialized. We infer that diet breadth had increased in younger, recently colonized populations. Preference diversification after colonization events, whether caused by (cryptic) host shifts or by release of cryptic genetic variation after population bottlenecks, provides a mechanism for known effects of range shifts on diet specialization. Our results explain how colonizations at expanding range margins have increased population-level diet breadths, and predict that increasing specialization should accompany population persistence as current range edges become range interiors.

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KEYWORDS

climate change, diet breadth, extinction–colonization dynamics, generalization, host shift, oviposition preference, range expansion, specialization

1 | INTRODUCTION

Range shifts caused by human manipulation of wild species' habitats and by regional climate warming are increasing in pace and prevalence (Chen et al., 2011; Cullingham et al., 2011; Lancaster, 2020; Parmesan 2006; Platts et al., 2019; Settele et al., 2014). These shifts influence latitudinal patterns of ecological specialization (Forister et al., 2015) by interacting in at least two ways with evolution along a specialist-generalist axis. One of these ways is both expected and shown: generalists should be better colonists than specialists, quicker to extend their ranges polewards as warming creates opportunity (Monaco et al., 2020; Platts et al., 2019). However, a second, less expected process, with the same result but the opposite direction of cause and effect, is also operating: the process of range expansion itself can cause evolution of generalism at the population level (Lancaster, 2020).

Using a global analysis of butterfly diets, distributions, and range dynamics, Lancaster (2020) showed that, in this taxon, the trend for broader diets at higher latitudes has been caused principally by range expansions themselves driving broadening of diets, rather than by expansions being preferentially undertaken by generalists. This effect of range shifts on diet breadth can complement the known tendency for large geographic range size to facilitate host shifts (Jahner et al., 2011). However, unlike the effect of range size, the effect on diet breadths of range expansion lacks an obvious mechanism. Here we reveal a candidate for the missing mechanism: the fine-scale interactions between colonizations and host shifts in our study insect, the butterfly, *Euphydryas editha*, generate an expectation of the cause–effect directionality shown by Lancaster (2020).

In addressing host shifts and diets breadths of herbivorous insects, we are fortunate that this topic has consistently fascinated evolutionary biologists for a century (Agosta, 2006; Brues, 1924; Ehrlich & Raven, 1964; Forister et al., 2012; Futuyama & Moreno, 1988; Jaenike, 1990; Larose et al., 2019; Thompson, 1998; Tilmon, 2008). The apparent conflict between the high degree of host specialization of most species (Ehrlich & Raven, 1964) and the rapid accumulation of insect communities on recently introduced plants (Strong, 1974) has been particularly intriguing (Agosta et al., 2010).

In recent decades, observed colonizations of exotic hosts have generated opportunities to investigate ongoing episodes of diet evolution in real time. Forister et al. (2013) found that individual Melissa Blue butterflies sampled from populations that had colonized an exotic host, alfalfa, were more generalized in their oviposition preferences than those sampled from populations still using their traditional native host. Hardy (2017) asked whether the processes that generate this type of pattern can be studied in

captive insects: “does experimental adaptation of a plant-eating insect population to a novel host result in host-use generalism, and improve the odds of evolving additional new host associations?” Braga et al. (2018) used an experiment “in silico” to answer this question in the affirmative.

Here we address these topics “in vivo” and in nature, applying a combination of long-term observations and experiments to a single butterfly species and illustrating relationships in real time between habitat colonizations, host shifts, population-level diet breadths, and heritable host preferences. Just as Braga et al. (2018) discovered in their computers, we show in wild populations that hosts whose use is opposed by natural selection have been briefly drawn into the diets of *E. editha* butterflies after colonization events and then, after a few generations, excluded again. We present evidence that this process is not confined to the populations in which we observed it, and that colonizations have caused expansions of diet breadth across our study species. This process can account for Lancaster's (2020) finding that range expansions, each of which comprises multiple colonizations, have likewise caused broadening of population-level diets.

2 | MATERIALS AND METHODS

2.1 | Study species

Edith's checkerspot butterfly (*E. editha*; *Nymphalidae*, *Melitaeinae*) uses different host genera in a geographic mosaic across its range (Singer, 1971; Singer & McBride, 2012; Singer & Wee, 2005). The insect occurs as isolated populations and metapopulations, both of which are typically localized, well-defined and subject to natural extinctions. This extinction–recolonization dynamic revealed one of the earliest examples of a modern range shift linked to anthropogenic climate warming (Parmesan, 1996). Adult *E. editha* lay eggs in clutches on hosts in the Orobanchaceae (*Pedicularis*, *Castilleja*) and Plantaginaceae (*Collinsia*, *Plantago*, *Penstemon*, *Veronica*, *Mimulus*, *Antirrhinum*). When the proportion of *E. editha* eggs laid on each host was ascertained by census at each of 57 sites, 43 populations were recorded as monophagous, with the remainder using two to four host genera (Singer & Wee, 2005). These populations showed strong isolation by distance but no isolation by host, so they did not comprise a set of host-associated cryptic species (Mikheyev et al., 2013). Figure 1 identifies the genus-level hosts used in the 1980s at each of the sites used in the genetic study reported here. We treat insects at each of these sites as “populations,” although some could equally be described as metapopulations. Dots without pie diagrams show sites where *E. editha* occurred that were not included in the current analyses.

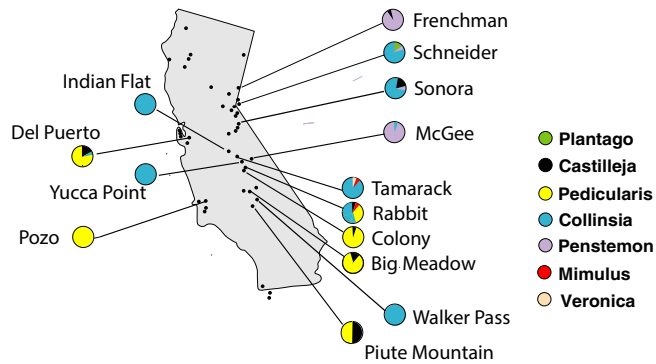


FIGURE 1 Snapshots of *Euphydryas editha* diet at the study sites in California and Nevada in the 1980s: results of censuses estimating proportions of eggs laid on each host genus at each site. The color-coding for host genera shown here is consistent through subsequent tables and figures. Identities to species level of hosts used at each site, and listing of potential hosts present but not used by the local butterflies, are given in Table S1; latitudes and longitudes are in Table S2.

2.2 | Oviposition preference-testing technique and diet-breadth censuses

Population-level diet breadths at oviposition were recorded by physically searching known hosts and potential hosts for eggs and first-instar or second-instar larvae, that is, larvae young enough that they could be assumed to be feeding on the host species that had received eggs. We searched each individual of scarce plant species and censused quadrats placed in a stratified-random design to estimate the proportional use by the butterflies of more abundant plants.

Behavioral tests of oviposition preferences were performed by capturing butterflies in the field and staging encounters with plants in their own habitats. Female *E. editha* that are motivated to oviposit behave naturally in staged encounters with potential hosts, allowing an experimenter to assess oviposition preferences by arranging a sequence of such encounters (Singer et al., 1992). These preference tests used a standardized experimental technique in which alternating encounters were staged between insect and plant. Plants were either left undisturbed in their natural habitats or freshly transplanted into pots in their own soil. Acceptance of plant taste was judged from full abdominal curling and extrusion of the ovipositor for 3 s. Acceptance and rejection were recorded at each encounter, but oviposition was not allowed (videos showing acceptance in such staged encounters are linked in McBride & Singer, 2010; Singer & Parmesan, 2019).

During each test, the range of plants that would be accepted, if encountered, expands over time with increasing motivation to oviposit. Therefore, acceptance of plant A followed by rejection of plant B is recorded as preference for A over B. The assumption that these insects' preferences are not influenced by prior experience, either as larvae or as adults, is supported by prior observation and experiment (McNeely & Singer, 2001). Testing of other assumptions underlying this technique is described in Singer et al. (1992). We used

two experimental designs, either testing each insect on the same individual plants or on different plants sampled independently from their populations; see footnote of Table 2.

2.3 | Relationship between population-level diets and host preferences of individuals

The use of the behavioral preference assay has shown that, in populations of *E. editha* using more than one host, this diversity of diet could be achieved either by weakness of oviposition preference, allowing butterflies to accept hosts that they did not prefer, and/or by diversity of preference rank within the population (Singer, 1983; Singer et al., 1989). Diversity of rank was an important source of diet variation within two populations, Rabbit Meadow and Schneider, where diet was known, from a combination of observation and experiment, to be rapidly evolving (Singer & Parmesan, 2018, 2019). In contrast, weakness of preference was the principal cause of diet variation in six populations that, judging from repeated censuses, were not indulging in bouts of diet evolution (Singer et al., 1994).

2.4 | mtDNA analyses

The original genetic study (Radtkey & Singer, 1995) included 24 populations of *E. editha* in California, of which 14 had sample sizes of $n = 4$ individuals or more and are included in the current analysis. Butterflies used in these analyses were sampled independently of the censuses used to assess diet breadths. We used 17 restriction endonucleases to identify 22 mtDNA haplotypes of *E. editha*, the distributions of which were recorded within and among 24 populations of the butterfly. This molecular technique is long outdated, but the data that it generated are not susceptible to bias for the questions we are asking, since we are not using them to aim at a target, such as a phylogeny. Instead, we use them as an index of genetic diversity, to ask whether an association existed between this index and diet breadth in the 1980s. For this purpose, our analysis is robust. It is particularly appropriate to use mtDNA, since it has half the effective population size of nuclear DNA, which should augment the effects of bottlenecks associated with population-founding events.

Haplotype diversity was itself diverse. Four populations were homogeneous, each containing only a single haplotype despite sample sizes of 11, 13, 17, and 30 individual insects, while at the opposite extreme one population in which 14 individuals were sampled produced seven haplotypes and a second population with a sample size of only four contained no replicates. Retention of this last informative sample was the reason for our choice of a sample size of four as the cutoff for analysis. Exclusion of populations with sample sizes of less than four reduced the number of populations from 24 to the 14 shown in Figure 1. We used a two-tailed Spearman rank test to ask whether population diet breadths were associated with the numbers of haplotypes found per individual sampled. The use of the per-individual statistic controls for variation of sample size.

3 | RESULTS

3.1 | Changes of diet and diet breadth: Long-term observations, 1980s–2010s

Detailed accounts of natural selection and behavioral mechanisms underlying evolution of diet and preference have been published for two of our study sites, Schneider's Meadow and the Rabbit metapopulation, each account filling an entire paper (Singer & Parmesan, 2018, 2019). This level of detail is beyond our current scope. Our dataset is heterogeneous: censuses were more frequent in some populations than in others, and we did not visit every population in every year. Here we summarize decadal changes for the set of populations reported here in order to focus on our primary question of the relationships between diet breadth and extinction/colonization dynamics.

Table 1 shows the time trends of diet breadth across decades in 15 populations for which we have long-term data on diet, 14 of which also provide data on mtDNA. The three left-hand columns of Table 1 show population names, the numbers of host genera on which *E. editha* eggs or larval webs were found during the 1980s, and the numbers of genera used in the most recent decade of observation, which, with four exceptions, was the 2010s.

Figure 2 complements Table 1, adding information on diet-breadth oscillations and extinction–colonization events. Populations where changes of diet breadth and/or extinctions have occurred are

identified in Figure 2 by two-letter or three-letter codes that link the information in the figure to that in Table 1. The timing of diet-breadth observations in Figure 2 is positioned by the decade in which they were made. Although, for some populations, data exist at greater accuracy than that, the overall trends are most easily seen at the decadal scale. Each skull and crossbones icon indicates both an extinction and a subsequent recolonization, and is not positioned by decade. It is placed between the last observation of diet breadth made before the extinction and the first observation made after the recolonization. There were often multiple censuses conducted after the initial extinction and prior to recolonization that are not shown in this figure.

Seven of the 15 study populations had the same diet breadth in the most recent census as in the 1980s while eight had narrower diets. None had broader diets. A two-tailed binomial test rejects the hypothesis that diet breadth was equally likely to have expanded or contracted ($p = 0.008$). Within our set of study populations, there has been a general trend for diet breadth to be reduced over time (caveats in Text S1).

3.2 | Diet breadths and preference diversities before and after colonizations

Here we provide details of changes at two sites where extinction/colonization events occurred and from which we have not only diet

Population	Number of host genera in 1980s	Number of host genera at last check	Number of mtDNA haplotypes	Sample size for mtDNA study (individuals)	mtDNA haplotypes/individuals
Rabbit Meadow RM	4 ¹	2	1	30	0.03
Sonora Junction SON	3 ²	2	2	4	0.50
Tamarack Ridge TR6	3 ²	1	2	14	0.14
Schneider Meadow SCH	3 ⁴	2	2	7	0.29
Del Puerto Canyon ^a DP	3 ²	2 (1990s)	2	14	0.14
Frenchman Lake FR	3 ²	2	1	13	0.08
Tuolumne Meadow	3 ²	1 (2000s)	n/a	n/a	n/a
Piute Mountain	2 ²	2	3	10	0.33
Colony Meadow	2	2	1	17	0.06
McGee Creek MG	2	1 (2000s)	1	11	0.09
Big Meadow	2 ³	2	4	19	0.21
Yucca Point YP	1 ³	1	3	7	0.43
Walker Pass	1 ³	1	4	4	1.00
Indian Flat	1 ^{2,3}	1	7	14	0.50
Pozo	1 ^{2,3}	1 (2000s)	2	6	0.33

Note: References: ¹Singer (1983), ²Singer et al. (1994), ³Radtkey and Singer (1995), ⁴Singer and Parmesan (2018). Where no reference is given, as is the case for most of the "last check" column, data are previously unpublished. Location of named sites shown in Figure 1.

^aErratum: Singer et al. (1994) recorded only two hosts at Del Puerto, forgetting to include *Collinsia bartsiaefolia*, which had not been used since the high-rainfall spring of 1983.

TABLE 1 Diet diversity over time for 15 study populations and mtDNA diversity for 14. Columns 2 and 3: maximum numbers of host genera used simultaneously at each site in the 1980s and in the most recent decade when the site was censused, which is the 2010s unless otherwise indicated. Columns 4–6: numbers of mtDNA haplotypes sampled, sample sizes, and number of haplotypes per individual sampled at each site in the 1980s. From Radtkey and Singer (1995). Hosts listed and identified to species in Table S1, lat-longs in Table S2

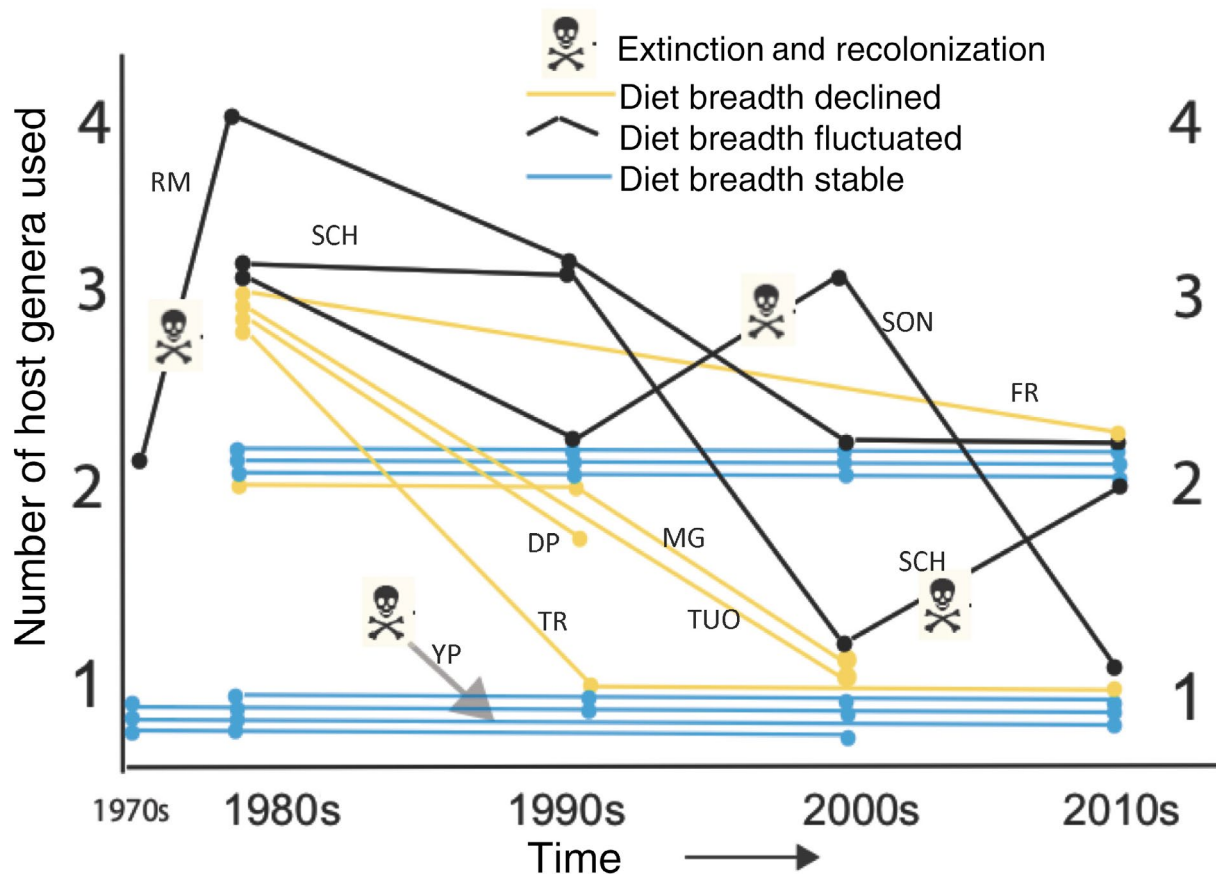
Changes of *Euphydryas editha* diet breadth across decades

FIGURE 2 Changes of *Euphydryas editha* diet breadth across decades. Population codes as in Table 1. Solid circles represent maximum diet breadths at each site for a given decade, usually representing results from multiple years. Censuses of eggs and young larvae were conducted as conditions permitted: all sites were censused at least once within each decade for which data are shown and some sites were censused multiple times/decade. Classification of a population as monophagous required a minimum sample size of 20 ovipositions (egg clusters or pre-diapause webs). There was greater variation in diet than evident on this figure: some sites had large shifts in proportions of eggs laid on the different host genera, but not in total number of genera used at the population level (this graphic). More detailed accounts of this type of change are beyond the scope of this paper. Sites not shown in the most recent survey were not censused in the 2010s. The skull and crossbones icon indicates that the population went extinct for at least a year and was subsequently recolonized, with the post-recolonization diet breadth indicated by the solid circle in the subsequent decade. See Sections 3.2.1, 3.2.2 and 4.8.2 for details of events at Sonora (SON), Rabbit (RM), and Schneider (SCH)

data but also experimental data from behavioral tests of individual female preferences, conducted both before the recorded extinction and after the subsequent colonization event. At each site, diet breadth expanded and contracted, but the mechanisms driving these dynamics were different in each case.

3.2.1 | Site: Sonora

When we first worked at Sonora (Figure 1) in the 1980s, host preference ranks were almost invariant; we found a single exception to the rule that butterflies either ranked *Castilleja pilosa*>*Collinsia parviflora*>*Penstemon rydbergii* or they showed no preference (Table 2). Experimental placement of eggs showed larval survival rates on the three hosts concordant with the rank order of insect preference: survival was highest on *Castilleja*,

lowest on *Penstemon* and intermediate on *Collinsia*. However, the top-ranked host, *Castilleja*, was estimated as receiving only 24% of the eggs laid, with *Collinsia* receiving 75% and *Penstemon* 1% (Singer et al., 1989). *Castilleja* was sufficiently rare that many searching insects failed to find it before reaching the level of oviposition motivation at which they would accept either *Castilleja* or *Collinsia*, whichever they encountered next. They were then more likely to encounter the more abundant host, *Collinsia*. The population achieved diet diversity principally by interaction between weakness of preference and rarity of the most preferred host (Singer et al., 1989).

The Sonora population underwent a natural extinction in the 1990s, was absent for about 4 years (confirmed with at least two intermediate censuses), and was recolonized by 1999. In 2002, we again conducted oviposition preference tests (Table 2; Figure 3). Preference ranks were diverse: we found all possible rank orders

for the three hosts, *Penstemon*, *Collinsia*, and *Castilleja*. Each of the three hosts was ranked by some individuals at the top of their preference hierarchy and by others at the bottom. As expected from these preference tests, population-level diet breadth at Sonora had increased in 2002: *Penstemon* had been added to the diet and all three hosts were substantially used. We found 20 egg clutches on *Castilleja* in a total census of this rare plant; 9 on *Collinsia* in a census covering approximately 40% of phenologically suitable plants and 14 on *Penstemon* in a census covering about 20% of these plants. We estimate that the most used host was *Penstemon*, which had previously been the least preferred of the three hosts and not used at all in some years.

Preference tests were once again performed at Sonora in 2014 and 2018 (Table 2; Figure 3). With the exception of two butterflies that preferred *Penstemon* over *Collinsia*, preferences had reverted to their original homogeneous ranking of *Castilleja*>*Collinsia*>*Penstemon*. We found no females that preferred *Penstemon* over *Castilleja* and only one, out of 50 tested, that failed to discriminate between these hosts, so it is not surprising that *Penstemon* had once again disappeared from the diet. Despite intensive censuses, in neither 2014 nor 2018 did we find a single oviposition on *Penstemon*. Both the diversification of preferences and the inclusion of *Penstemon* into the diet as a major host had been ephemeral, appearing rapidly following the recolonization event, then disappearing just as quickly.

3.2.2 | Site: Rabbit

Prior to human intervention, *E. editha* in the Rabbit metapopulation (represented as "Rabbit" in Figure 1 and as "RM" in Figure 2) used two perennial hosts and occupied >20 habitat patches distributed across 8 × 10 km (Singer & Thomas, 1996). The principal diet was the perennial *Pedicularis semibarbata*, with minor use of the much rarer *Castilleja disticha* (Singer & Parmesan, 2019). Two potential hosts, both ephemeral annuals, were present but not used: the super-abundant *Collinsia torreyi*, and the rare *Mimulus whitneyi*. Natural selection opposed using *Collinsia* despite its abundance because its lifespan was so short at this site that larvae

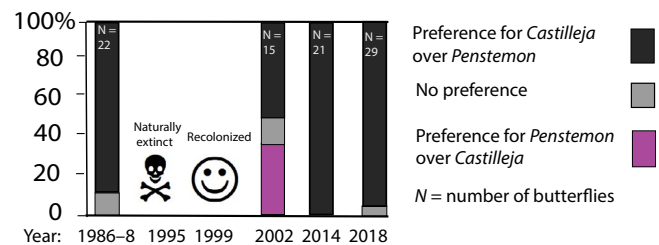


FIGURE 3 Proportions of butterflies preferring *Castilleja* or *Penstemon* at Sonora before and after natural extinction and recolonization (additional data in Table 2). Host diet color-coded by genus as in Figure 1

		<<<<Prefer plant named at left	No preference	Prefer>>>> plant named at right	
1986-1988	<i>Castilleja</i>	20	2	0	<i>Penstemon</i>
1986-1988	<i>Castilleja</i>	13	9	0	<i>Collinsia</i>
1986-1988	<i>Collinsia</i>	43	3	1	<i>Penstemon</i>
Extinction and recolonization					
2002	<i>Castilleja</i>	7	1	6	<i>Penstemon</i>
2002	<i>Castilleja</i>	5	5	5	<i>Collinsia</i>
2002	<i>Collinsia</i>	12	2	10	<i>Penstemon</i>
2014	<i>Castilleja</i>	21	0	0	<i>Penstemon</i>
2014	<i>Castilleja</i>	21	0	0	<i>Collinsia</i>
2014	<i>Collinsia</i>	24	5	0	<i>Penstemon</i>
2018	<i>Castilleja</i>	28	1	0	<i>Penstemon</i>
2018	<i>Castilleja</i>	29	1	0	<i>Collinsia</i>
2018	<i>Collinsia</i>	18	5	2	<i>Penstemon</i>

TABLE 2 Preference ranks at Sonora before and after natural extinction and recolonization. Host diet color-coded by genus as in Figure 1

Note: Footnote to Figure 3 and Table 2: data from 1986 to 1988 from Singer and Parmesan (1993), Singer et al. (1989). Data from 2002, 2014, and 2018 previously unpublished. Host diet color-coded by genus as in Figure 1. We used two experimental designs: (1) tests in which all butterflies were offered the same individual plants, to reveal variation among individual butterflies and (2) tests in which each butterfly was offered a different set of individual hosts, to allow for effects of variable acceptability within host populations, which can generate apparent variability in the identity of the host species that is preferred (Singer & Lee, 2000; Singer et al., 2002). Data shown from 2002 are those obtained from the first design; the butterflies were truly variable. In 2014 and 2018, we used both designs but show results from the second design, which is conservative to the result, maximizing the likelihood of recording diverse preferences.

hatching from eggs laid on it were almost certain to starve by failing to reach diapause before host senescence (Moore, 1989; Singer & McBride, 2012).

Starting around 1967, humans made 18 clearings in which all trees were removed, fires were set and ground was bulldozed, locally extirpating the butterflies from the cleared areas. The effect of fertilization from the fires extended the size and lifespan of *Collinsia* to the point where it could accommodate the life cycle of the butterflies. *Collinsia* in clearings suddenly became a benign environment for the larvae, supporting higher fitness than the well-defended *Pedicularis*, despite the butterflies being adapted to *Pedicularis* and demonstrably maladapted to *Collinsia* in a suite of host-adaptive traits (Singer & Parmesan, 2019).

The skull and crossbones along the RM line in Figure 2 represent the anthropogenic local extinction caused by clear-cut and burn in the single "Rabbit Meadow" clearing, a large (>2 ha) clearing within the "Rabbit" metapopulation where we have studied diet intensively across the decades. By 1979, the clearing had been colonized by butterflies immigrating from adjacent unlogged patches, where the insects had persisted on their original diet of *Pedicularis* and *Castilleja*. In 1981, a detailed census and map was made of the distribution of *E. editha* ovipositions in the clearing. Eggs had been laid on four hosts: two novel hosts, *Collinsia* and *Mimulus*, plus the two traditional hosts, *Pedicularis* and *Castilleja* (Singer, 1983). *Pedicularis* is a hemiparasite of gymnosperms, killed by logging, so it was restricted to the margins of the clearing. *Collinsia* and *Mimulus* were used in the center of the clearing but remained unused in the adjacent unlogged patch, where both occurred and *Collinsia* was abundant. This pattern of host use sets the context for the two cases of preference diversification that occurred in the Rabbit Meadow clearing during the 1980s and that are detailed in Sections 3.2.2.1 and 3.2.2.2.

In the 1990s, the direction of natural selection across the Rabbit metapopulation was reversed, favoring preference for *Pedicularis* over *Collinsia*. The direction of evolution reversed in response, and the use of *Collinsia* diminished until this host was permanently abandoned in 2001. Since then the metapopulation has reverted to its ancestral diet of *Pedicularis* and *Castilleja*, with butterflies concentrated in the unlogged patches and rare in the clearings (Singer & Parmesan, 2019).

2.2.1 | Adaptive diversification of preference as part of host shift from *Pedicularis* to *Collinsia*

Butterflies in Sequoia National Park (c.12 km from Rabbit) represent the putative pre-logging state of the Rabbit metapopulation. We found no diversity of preference rank; most butterflies from the Park showed varying strengths of preference for *Pedicularis* over *Collinsia* and a few showed no preference, but none preferred *Collinsia* over *Pedicularis* (Singer & Thomas, 1996).

In contrast, preference ranks for the same two hosts in the anthropogenically altered Rabbit Meadow clearing were diverse and evolving through the 1980s. In the early 1980s, most insects emerging in the center of the clearing preferred to oviposit on *Pedicularis*, despite having developed on *Collinsia* from eggs naturally laid on it. The proportion of these *Collinsia*-emerging butterflies that preferred *Collinsia* increased significantly between 1984 and 1989 (Singer & Thomas, 1996). This increase in preference for *Collinsia*, and the diversification of preference from the starting condition lacking diversity of preference rank, is consistent with adaptive evolutionary response to measured natural selection that favored preference for *Collinsia*, but that acted on an initially *Pedicularis*-preferring population (Singer & Parmesan, 2019).

2.2.2 | Non-adaptive preference diversification: Incorporation of *Mimulus* into the diet as a side effect of host shift to *Collinsia*

In the ancestral state, *Mimulus* and *Collinsia* were present but neither was used for oviposition, though *Collinsia* was fed upon by wandering late-instar larvae. In 1981–1982, following logging and burning, both hosts were used in the clearing and oviposition preferences for them were diverse (Table 3b). Field experiments (Singer et al., 1994) estimated larval survival on *Collinsia* in the clearing as three times higher than that on *Mimulus* (*Mimulus* is "host 4" in figure 2 of Singer et al., 1994). Natural selection favored using *Collinsia* but not *Mimulus*. *Mimulus* had been included in the diet despite natural selection against using it, but this situation proved short-lived: by 1988, preferences for *Collinsia* over *Mimulus* had become homogeneous and *Mimulus* was no longer used (Tables 3a and 3b). We found the same preference homogeneity again in 2019, but the experiment was not strictly comparable. We used butterflies from

TABLE 3A Egg distributions on *Collinsia* and *Mimulus* in Rabbit Meadow clearing and adjacent unlogged patch. Each quadrat was 30 cm × 30 cm and could contain several hundred individual *Collinsia* plants and >1 egg clutch

Year	Habitat: clearing patch		Habitat: unlogged patch	
	Clutches on <i>Collinsia</i> /quadrats searched	Clutches on <i>Mimulus</i> /plants searched	Clutches on <i>Collinsia</i> /quadrats searched	Clutches on <i>Mimulus</i> /plants searched
1979	16/41	^a	0/22	^a
1981	5/33	6/25	0/50	0/32
1982	37/118	13/36	0/56	0/46
1988	58/50	0/47	0/20	0/18
1989	9/69	0/37	0/25	0/35
1991	19/54	0/18	0/20	0/22
2019	0/40	0/13	0/25	0/61

^aNo census was done.

TABLE 3B Preferences for *Collinsia* vs. *Mimulus* at Rabbit Meadow

Year	Butterflies preferring <i>Collinsia</i>	No preference	Butterflies preferring <i>Mimulus</i>
1981–1982 (clearing)	11	12	8
1988–1992 (clearing)	23	3	0
2019 (unlogged patch)	22	0	0

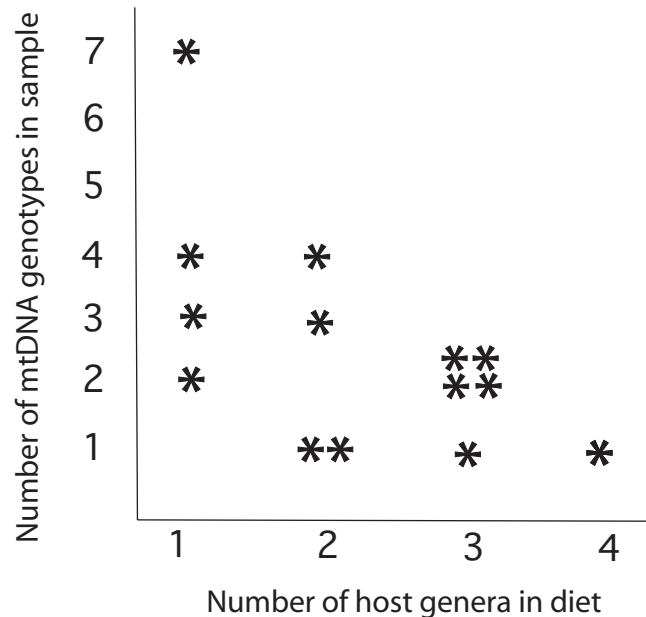


FIGURE 4 Numbers of mtDNA haplotypes found in the 14 study populations of *Euphydryas editha* plotted against the 1980s' diet breadths shown in Figure 1 and Table 1

the unlogged patch adjacent to the Rabbit Meadow clearing rather than from the clearing itself, since *Collinsia* had not been used as an oviposition host since 2001, and butterflies in the clearing were scarce (Section 3.2.2).

3.3 | Genetic evidence: mtDNA and diet breadth

Re-examination of the mtDNA dataset first published long ago (Radtkey & Singer, 1995) reveals a relationship on which the original study did not comment: an inverse association between population-level diet breadth in the 1980s and mtDNA diversity (Figure 4; Table 1); samples from populations using fewer host genera contained more mtDNA haplotypes.

Because sample sizes were diverse, the association shown in Figure 4 might have stemmed from sampling more individuals from populations that happened to be monophagous than from those with broader diets. However, the opposite was the case: a regression of mtDNA sample sizes on diet breadth, using the data in Table 1, gives a slope of +3.6 ($p = 0.06$, two-tailed). The direction of this trend, with higher mtDNA sample sizes from populations with broader diets, is

opposite to that expected to produce the relationship in Figure 4. To control for sample size in testing significance of the genetic diversity/diet-breadth relationship, we calculated an estimate of mtDNA diversity as the number of haplotypes per individual sampled (Table 1, right-hand column). The association between this statistic and the diet breadths listed in column 2 of the table is significant with $p = 0.024$, by Spearman rank test (two-tailed).

4 | DISCUSSION

We exploit our study insect's extensive ecotypic variation and penchant for rapid evolution of host preference to document a novel behavioral mechanism underlying Lancaster's (2020) finding that butterfly range expansions have caused loss of population-level dietary specialization. In species that resemble *E. editha* in the mechanics of their diet evolution, colonizations at expanding range margins will increase both diversity of host preferences and population-level diet breadths. At the same time, increasing specialization will accompany population persistence in range interiors.

Following extinctions of two populations in the interior of *E. editha*'s range, diets recorded after recolonization were broader than they had been at the same sites in pre-extinction populations (Section 3.1; Figure 2). We can generalize this effect of colonizations on diet breadth at least to our study species as a whole, since we show a significant negative association among populations between mtDNA diversity and diet breadth, suggesting that young populations had broad diets and low genetic diversity while older ones had acquired greater genetic diversity and evolved local adaptation in the form of greater specialization (Section 3.3; Table 1; Figure 4).

Behavioral preference tests administered in the field showed that the principal mechanism of the post-colonization diet broadening events that we observed was not loss of specialization by individuals, but diversification of specialists (Section 3.2; Figure 3; Tables 2 and 3b). Variability of oviposition preferences had increased in the newly recolonized populations. What might have caused this diversification? Two different, non-exclusive, processes are likely, which we discuss in Sections 4.5 and 4.6. First, the population bottleneck associated with the colonization may reveal previously cryptic additive genetic variance (Hoffmann et al., 2017; Paaby & Rockman, 2014; van Heerwaarden et al., 2008). Second, colonizations may be accompanied by host shifts and it may be the host shifts that cause preferences to diversify.

4.1 | Oviposition preferences of *E. editha* are heritable

Past work, reviewed by Singer and Parmesan (2019), has shown that oviposition preferences of our study insect are heritable and unaffected by experiences of larvae or adults. Two sets of experiments were performed. First, preferences of freshly caught females were measured within a single season at a single site, Schneider, and offspring were raised in the "laboratory"—a greenhouse—on a common

host. Individual laboratory-raised daughters were preference-tested "blind," in that the tester did not know the identities of their mothers or sibs. The daughters resembled their mothers in preference, with $p < 0.005$ and $r = 0.45$ with 95% CI 0.16–0.74 (Singer et al., 1988). Given that male contribution in this experiment was random and that male and female contributions to inheritance of oviposition preference were later shown to be approximately equal (McBride & Singer, 2010), we can estimate heritability of preference as twice the mother-daughter correlation, with the lower bound of the 95% CI at $h^2 = 0.32$.

The second set of experiments comprises measurements made at Schneider while rapid changes of preference were occurring in nature. In 2 years, 1983 and 1990, we measured preferences of freshly captured butterflies for the exotic *Plantago* versus their traditional *Collinsia* host. We also measured preferences of offspring of 1983 butterflies and 1990 butterflies that we had raised on *Collinsia* in a greenhouse. Changes of preference between the 2 years were significant and similar in the field-caught and laboratory-raised butterflies, indicating that the change in preference measured in nature was evolutionary (Singer et al., 1993).

4.2 | Long-term observations of increasing specialization: Evidence for independence of data

Seven of the 15 populations in our study used three or four host genera in the 1980s, but by the 2010s none used more than two. In using a statistical test to reject the hypothesis that specialization was equally likely to increase or decrease, we assume that changes of diet at different sites were independent events. Our justification for this assumption is that *E. editha* is a sedentary butterfly (Ehrlich, 1961) with a maximum recorded movement of 5.6 km (Harrison, 1989). Observed evolution of host preference has differed between populations separated by <10 km and appeared independent between populations 12 km apart (Singer & Thomas, 1996). No two populations in the current study underwent the same changes of diet. We therefore assume that our study populations, scattered across California as they are (Figure 1), were evolving independently of each other over the decadal timescales that we used and that the long-term census data, showing an overall trend for increasing specialization across four decades, are not effectively pseudoreplicated.

4.3 | Two colonizations followed by diversification of preferences and expansions of diet breadth

Colonizations at the Rabbit Meadow clearing and at Sonora were followed by increases in population-level diet breadth that were shown by experiment to be driven by within-population diversification of oviposition preference. At Sonora, a natural extinction-colonization event was followed in 2002 by an explosion of preference diversity (Section 3.2.1; Table 2; Figure 3) and expansion of diet breadth in which a formerly avoided plant, *Penstemon*, became the principal

host. Experiment had previously shown natural selection against use of *Penstemon* at this site (Singer et al., 1994). In the latest censuses, in 2014 and 2018, *Penstemon* was no longer used and preferences for the most preferred host, *Castilleja*, over *Penstemon* were homogeneous.

At Rabbit Meadow, colonization of a habitat patch in which humans had extirpated the butterflies was accompanied by addition of a novel host, *Collinsia*, that supported high fitness due to extended longevity after anthropogenic fire. This host shift was favored by natural selection (Singer & Parmesan, 2019), but the adoption of *Collinsia* was accompanied by addition to the diet of a second novel host, *Mimulus* (Table 3a), that did not support high fitness but was preferred for oviposition by some butterflies (Table 3b). Selection opposed the use of *Mimulus* (Singer et al., 1994) and it was abandoned within a decade (Section 3.2.2; Table 3a; shown in Figure 2 as the decline from 4 to 3 genera at population RM), contributing to the overall trend for increasing specialization as populations persisted.

We admit to being puzzled by the speed with which diet-breadth expansions have been followed by returns to specialization. Even though oviposition preferences of *E. editha* are highly heritable and we have estimated strong natural selection on host use (Moore, 1989; Singer et al., 1994), returns to specialization have been unexpectedly fast: less than 12 generations at Sonora (Table 2) and less than 6 for the loss of preference for *Mimulus* over *Collinsia* at Rabbit Meadow (Table 3b).

4.4 | Genetic evidence supports generality of diet-breadth expansions following colonizations

In the 1980s, populations with broader diets had significantly lower mtDNA diversity than specialist populations using fewer host genera (Section 3.3; Figure 4; Table 1). We expect these mtDNA data to be subject to founder effects in the same manner as data gathered by more modern techniques. Therefore, a negative association between variability of diet and of genotype is expected if population-founding events frequently caused expansions of diet breadth like those we observed in real time. Young populations would have broader diets and reduced genetic diversity from founder effects; older populations would have evolved specialization as a local adaptation while acquiring genetic diversity. Newly founded populations are, indeed, expected to have reduced genetic diversity and to acquire more genotypes as they age, from some combination of immigration and mutation (Austerlitz et al., 1997; Excoffier et al., 2009). However, it is not obvious that recently colonized populations should have broader diets than the sources from which they were derived. We address this question in Section 4.6.

4.5 | Two ways for host shifts to cause diversification of preferences

Host shifts clearly diversify preferences when, during a shift, individuals retaining preference for the traditional host occur in the

same population and at the same time as those preferring the novel host. This was the case both for the shift from *Pedicularis* to *Collinsia* at Rabbit Meadow (Section 3.2.2.1) and for the shift from *C. parviflora* to *Plantago lanceolata* at Schneider (Singer & Parmesan, 2018).

A different possible role for host shifts stems from the evolutionary dimensionality of preference. Evolutionary transitions from traditional to novel hosts may cause additional, unexpected hosts to be drawn into to an insect's diet even if this addition is opposed by natural selection. This hypothesis was put forward by Hardy (2017) and supported in a model by Braga et al. (2018). We illustrate two examples: the apparently temporary use of *P. rydbergii* by the Schneider population during its host shift from *Collinsia* to *Plantago* (Singer & Parmesan, 2018) and the clearly temporary use of *Mimulus* by the Rabbit Meadow population in the early stages of its host shift from *Pedicularis* to *Collinsia* (Section 3.2.2, Tables 3a and 3b; Singer & Parmesan, 2019). In both cases, we estimated that use of the unexpected host was opposed by natural selection and both the unexpected hosts were eliminated from the diets after only a few generations.

4.6 | Two ways for colonizations to cause diversification of preferences: bottlenecks and host shifts

4.6.1 | Bottlenecks

Cryptic genetic variation that exists in natural populations can be revealed by changing conditions (Hoffmann et al., 2017; Paaby & Rockman, 2014). For example, experimental application of population bottlenecks to *Drosophila bunnanda* revealed cryptic genetic variation for desiccation resistance, causing an increase in additive genetic variance for this trait (van Heerwaarden et al., 2008). This is not an isolated result; bottlenecks frequently augment additive genetic variance, in apparent opposition to expected effects of genetic drift (Cheverud & Routman, 1996; Taft & Roff, 2012). We see a possible parallel between the result of the experiments with *D. bunnanda* and the increases in preference diversity that followed colonization events in our study of *E. editha*.

4.6.2 | Association between colonizations and cryptic host shifts

If colonizations tended to cause host shifts, the host shifts could then diversify preferences as we described in the previous section. Overall, it seems unlikely that colonizing female *E. editha* would switch host genera with sufficient frequency to produce the association in Figure 4. However, colonizations might routinely involve host shifts if those shifts were, from the butterflies' perspective, more frequent than changes of host genus that are observable to humans. We suspect this to be the case. Colonizations that do not shift host species will always involve changes of host population, and variation among conspecific host populations can be extremely important to butterflies (Harrison et al., 2016), including Melitaeines, the subfamily containing *Euphydryas*. Prior studies of discriminations made by Melitaeine

butterflies within and among host species have shown that, from the perspectives of all three butterfly species investigated (*E. editha*, *Euphydryas aurinia* and *Melitaea cinxia*), variation of acceptability among host individuals or conspecific populations was equivalent in magnitude to variation among host genera (Singer & Lee, 2000; Singer & Parmesan, 1993; Singer et al., 2002; see Glossary for definitions of "preference" and "acceptability" and Text S2 for descriptions of experiments and their results). Because variation among conspecific host populations is so important to Melitaeines, it will often be the case that a colonizing female is effectively undertaking a host shift even if the host she uses after migrating is the same species on which she developed at her site of origin.

If each host population were effectively unique from the butterflies' perspective, then adapting to a newly colonized population of a traditional host species could cause additional host species to be temporarily drawn into the diet, just as if the novel and traditional hosts were different species. Although the underlying mechanism by which such "drawing in" might occur has not been elucidated, our data suggest that it exists (Section 4.5).

4.7 | Rejection of gene flow and drift as causes of observed diet-breadth variation

A population may achieve a broad diet by containing a diversity of host-adapted genotypes. If such a population sends out colonists that found new populations, those populations should, through founder effects, have narrower diet breadths than their source. This process has been called "specialization by drift" and there is phylogenetic evidence that it has been important in scale insects (Hardy et al., 2016). However, if it were important in *E. editha* we would expect young populations to have narrower diets than established ones, the opposite of the inference that we draw from our results.

As gene flow continues after population founding, established populations receiving more diverse gene flow from multiple sources might become the ones with the broadest diets. However, if this process were driving interpopulation variation, we would expect a positive association between genetic diversity and diet breadth, the opposite of our current findings (Section 3.3; Table 1; Figure 4). Gene flow and founder effects are not implicated as causes of the variable diet breadths in our study system.

4.8 | Specialization is not an evolutionary dead-end, either over millions of years or decades; specialized populations contain cryptic variation of preference susceptible to genetic assimilation

4.8.1 | Phylogenetic analyses

These have tested the plausible hypothesis that specialists are derived from generalists more frequently than evolution in the opposite direction, and that specialization can be an evolutionary dead-end.

This hypothesis was not supported (Forister et al., 2012; Janz et al., 2001; Nosil, 2002); phylogenetic analyses indicate that diet breadth evolves readily in either direction. The idea that this bidirectional evolvability causes oscillations between specialization and generalization, and that these oscillations have acted as important drivers of insect speciation and biodiversity, first emerged from analyses of the butterfly family Nymphalidae (Janz et al., 2001, 2006). This idea has stimulated lively and apparently unresolved debate (Braga et al., 2018; Hamm & Fordyce, 2016; Hardy, 2017; Janz et al., 2016; Nylin et al., 2018).

4.8.2 | Three real-time observations of diet-breadth oscillation

We observed, in real time, three miniature versions of the diet-breadth oscillations deduced from phylogenetic analyses. The oscillations at Sonora and Rabbit Meadow were described in Sections 3.2.2.1 and 3.2.2.2. The third example is the Schneider host shift, which we did not include in Section 3 under "Results" since we present no new data. The most likely starting point was monophagy on *Collinsia*, since that was the diet of the nearest-known population of the same *E. editha* ecotype, at Curtz Lake, when the exotic *Plantago* had not yet arrived (Thomas et al., 1987). In preference trials administered on site at Curtz Lake, no butterflies preferred *Plantago* over their own *Collinsia* host, though around 10% had no preference (Thomas et al., 1987). Assuming that this represents the initial condition at Schneider, we conclude that, during its host shift from *Collinsia* to *Plantago*, the population passed from absence of preference for *Plantago* over *Collinsia* prior to the start of our study, through the phase in 1982–1990 when both preferences and diet were diverse, to eventually achieve monotonous preference for *Plantago* over *Collinsia*, monophagy on *Plantago* and abandonment of the traditional host in 2002 and 2005 (Singer & Parmesan, 2018).

4.8.3 | Cryptic preference variation and genetic assimilation

Although we judged the initial diet of the Schneider population to be both monotonous and monophagous (Section 4.8.2), the population contained variation of preference which was cryptic, in the sense that it had no effect on diet until it was revealed by the arrival of the exotic *Plantago*. From this point, we can imagine increasing preference for *Plantago* evolving by a classic genetic assimilation process (Paaby & Rockman, 2014).

As at Schneider, the starting condition for diet evolution in the Rabbit Meadow population was absence of preference for the novel host *Collinsia*, with variable strengths of preference for *Pedicularis* over *Collinsia* and a few individuals without preference (Section 3.2.2.1). Again, this variation was cryptic until humans, by logging, killed the *Pedicularis* (which parasitizes trees) and created large

patches of *Collinsia* from which individual butterflies with weaker preferences were unable to escape before reaching the oviposition motivation at which they would accept *Collinsia* (Singer & Parmesan, 2019). The result was the creation of booming populations in clearings that used *Collinsia* but in which most individuals retained preference for *Pedicularis*. Natural selection for acceptance of *Collinsia* was strong in those populations and evolution of preference was rapid (Singer & Thomas, 1996).

Even in monophagous *E. editha* populations in which all individuals show the same preference rank and none are without preference, preferences are not invariant, since the strength of preference is still variable: individuals differ in the length of the fruitless search that they would undertake before accepting a low-ranked host (Singer, 1982). As in the Rabbit Meadow example just described, it is possible for this variation to be revealed and exposed to selection when, for whatever reason, butterflies fail to find their preferred host.

4.9 | Contrary results to ours: Colonizations cause specialization

In contrast to our results, two studies on other species have shown increased dietary specialization after colonization. Hardy et al. (2016) use phylogenetic analyses to argue that, in scale insects, diet diversity is positively associated with genetic diversity, so founder effects associated with colonizations and range expansions have caused population-level diet to become more specialized, not less. Again conversely to our own results, the poleward range expansion of the Brown Argus butterfly (*Aricia agestis*) in the UK has been associated with increasing host specialization. In the expanding parts of the range of this insect, oviposition preferences were more specialized and homogeneous, both within and among populations, than in regions where the insect was long established (Bridle et al., 2014). In addition, larvae in the expanding regions were physiologically more host specialized and had lost evolvability, compared to their ancestral populations (Buckley & Bridle, 2014).

These contrary results give us pause in suggesting the level of generality of our result that colonizations cause loss of specialization. However, the ability of our results to help explain both the global latitudinal pattern of specialization documented by Forister et al. (2015) and the cause–effect relation between range shifts and diet breadth documented by Lancaster (2020) suggests that the mechanisms that we document here are not unique.

4.10 | Implications of our study for ecological speciation

There are none. Much of the literature that ties insect diet evolution to generation of biodiversity carries the assumption that host shifts facilitate speciation. In Melitaeine butterflies, this does not seem to be true. Host shifts are frequent, closely related sympatric insect species typically have overlapping diets (LaFranchis, 2004), and *E. editha* itself

shows strong isolation by distance but no residual isolation by host (Mikheyev et al., 2013). The failure of Melitaeines to speciate with host shift may reflect the fact that they do not mate on their hosts. Apart from this trait, we have no reason to think that diet evolution in Melitaeines is unusual, so we expect its mechanisms, as revealed in the current study, to be informative about processes that operate more widely than in this butterfly subfamily. Whether the short-term changes we show are informative about long-term diet-breadth oscillations (Braga et al., 2018; Hamm & Fordyce, 2016; Hardy, 2017; Janz et al., 2001, 2006, 2016) is an open question, but the fact that insects tend to recolonize long-lost ancestral diets suggests that processes measured on very different time scales are related.

4.11 | Contribution to understanding relations between range shifts and diet evolution

The processes that we document here support the cause-effect directionality of range-shift effects on diet breadth shown by Lancaster (2020) and help to account for the global latitudinal pattern of specialization: the trend for temperate zone species to be less specialized than those in the tropics (Forister et al., 2015; Monaco et al., 2020). As more and more species track shifting climate spaces driven by current warming trends, the numbers experiencing poleward range expansions will continue to rise. Yet, we have little understanding of the behavioral and evolutionary processes accompanying these ecological range expansions. The mechanisms driving diet expansion and contraction that we document here are novel, especially the finding that increases in generalization at the population level can stem from diversification of specialist individuals rather than from each individual becoming more generalist.

These results help us to better understand underlying dynamics operating at range boundaries and during extinction/colonization episodes. In addition to elucidating associations between colonizations, host shifts, and range shifts, our analyses of the heritability, dimensionality, and evolutionary agility of host preferences contribute to a mechanistic understanding of insect diets and host shifts in general. Incorporating these details into our understanding will better inform projection models and conservation planning under continued anthropogenic climate change.

GLOSSARY

Population-level diet breadth: In the studies reported here, the number of host species on which eggs of *E. editha* were laid in a particular population.

Host use: Again, in the work reported here, the proportion of eggs laid on each host species by an insect or an insect population. In a practical sense, this must most often be measured from the distributions of silken webs spun by young larvae, although groups that do not survive to this stage are missed by this technique (see Section 2).

Acceptance: A positive behavioral response by an insect to an encounter with a plant. It is a description of an observable and measurable event. It is not a trait of either plant or insect, since it depends on both insect preference and plant acceptability (see below). It is a trait of the plant–insect interaction (Singer, 2000).

Insect preference: The set of likelihoods of accepting particular specified hosts that are encountered. Defined in this way, it is a property of the insect that can vary among individuals (Singer, 2000) and can be heritable. *E. editha* first encounters hosts visually, then chemically, then physically, with separate preferences expressed at each stage (Singer & Parmesan, 2019). Again, in *E. editha*, the strength of post-alighting preference for two hosts, say host A and host B, is measured by the length of time that a female will search accepting only host B (if encountered) until, after failing to find host B, she reaches the level of oviposition motivation at which either A or B would be accepted, whichever is next encountered (details and justification in Singer et al., 1992).

Plant acceptability: The set of likelihoods that a plant will be accepted by particular specified insects that encounter it. Defined in this way, it is a property of the host that can vary among individuals (Singer, 2000) and can be heritable (Singer & Parmesan, 1993).

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CONFLICT OF INTEREST

The authors declare absence of competing financial interests.

AUTHOR CONTRIBUTION

Both authors participated in field censuses, oviposition tests, and writing. MCS performed the statistical analyses.

DATA AVAILABILITY STATEMENT

Relevant data are provided in Tables and in online Supporting Information.

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SUPPORTING INFORMATION

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